

WE CLAIM:

1. A method of detecting RNA polymerase activity in a continuous-read manner, comprising the steps of:
  - 5 (a) contacting an RNA polymerase with an oligonucleotide template in a reaction mixture comprising an assay buffer, under conditions in which the RNA polymerase is active;
  - (b) adding a fluorescent dye capable of binding double-stranded nucleic acid molecules to the reaction mixture;
  - 10 (c) measuring the fluorescence of the reaction mixture.
2. The method of claim 1, wherein the RNA polymerase is a recombinant RNA polymerase.
- 15 3. The method of claim 1, wherein the RNA polymerase is the Hepatitis C virus (HCV) polymerase, NS5B.
4. The method of claim 3, wherein the NS5B polymerase is a recombinant NS5B polymerase.
- 20 5. The method of claim 4, wherein the recombinant NS5B polymerase comprises an amino acid sequence as set forth in SEQ ID NO: 2.
6. The method of claim 5, wherein the reaction mixture further comprises large unilamellar vesicles.
- 25 7. The method of claim 4, wherein the recombinant NS5B polymerase comprises an amino acid sequence as set forth in SEQ ID NO: 4.
- 30 8. The method of claim 1, wherein the fluorescent dye is an unsymmetrical cyanine fluorescent dye.

9. The method of claim 8, wherein the unsymmetrical cyanine fluorescent dye is excited at between 475 nm and 495 nm and dye fluorescence is detected at between 518 nm and 542 nm.
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10. A method of detecting RNA polymerase activity in a continuous-read manner, comprising the steps of:
- (a) contacting an RNA polymerase with an oligonucleotide template in a reaction mixture comprising an assay buffer and a fluorescent dye capable of binding double-stranded nucleic acid molecules, under conditions in which the RNA polymerase is active; and
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- (b) measuring the fluorescence of the reaction mixture.
11. The method of claim 10, wherein the RNA polymerase is a recombinant RNA polymerase.
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12. The method of claim 10, wherein the RNA polymerase is the Hepatitis C virus (HCV) polymerase, NS5B.
13. The method of claim 12, wherein the NS5B polymerase is a recombinant NS5B polymerase.
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14. The method of claim 13, wherein the recombinant NS5B polymerase comprises an amino acid sequence as set forth in SEQ ID NO: 2.
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15. The method of claim 14, wherein the reaction mixture further comprises large unilamellar vesicles.
16. The method of claim 13, wherein the recombinant NS5B polymerase comprises an amino acid sequence as set forth in SEQ ID NO: 4.
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17. The method of claim 10, wherein the fluorescent dye is an unsymmetrical cyanine fluorescent dye.

5 18. The method of claim 17, wherein the unsymmetrical cyanine fluorescent dye is excited at between 475 nm and 495 nm and dye fluorescence is detected at between 518 nm and 542 nm.

19. A method of screening for compounds that modulate RNA polymerase activity in a continuous-read manner, comprising the steps of:

- 10 (a) contacting an RNA polymerase with an oligonucleotide template in a reaction mixture comprising an assay buffer, under conditions in which the RNA polymerase is active;
- (b) adding a fluorescent dye capable of binding double-stranded nucleic acid molecules to the reaction mixture;
- 15 (c) adding a test compound to the reaction mixture;
- (d) measuring the fluorescence of the reaction mixture; and
- (e) determining whether the test compound modulates RNA polymerase activity.

20 20. The method of claim 19, wherein the RNA polymerase is a recombinant RNA polymerase.

21. The method of claim 19, wherein the RNA polymerase is the Hepatitis C virus (HCV) polymerase, NS5B.

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22. The method of claim 21, wherein the NS5B polymerase is a recombinant NS5B polymerase.

23. The method of claim 22, wherein the recombinant NS5B polymerase comprises an amino acid sequence as set forth in SEQ ID NO: 2.

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24. The method of claim 23, wherein the reaction mixture further comprises large unilamellar vesicles.
25. The method of claim 22, wherein the recombinant NS5B polymerase  
5 comprises an amino acid sequence as set forth in SEQ ID NO: 4.
26. The method of claim 19, wherein the fluorescent dye is an unsymmetrical cyanine fluorescent dye.
- 10 27. The method of claim 26, wherein the unsymmetrical cyanine fluorescent dye is excited at between 475 nm and 495 nm and dye fluorescence is detected at between 518 nm and 542 nm.
- 15 28. The method of claim 19, wherein the compound that modulates the RNA polymerase activity is an antagonist of the RNA polymerase activity.
29. The method of claim 19, wherein the compound that modulates the RNA polymerase activity is an agonist of the RNA polymerase activity.
- 20 30. A method of screening for compounds that modulate RNA polymerase activity in a continuous-read manner, comprising the steps of:
- (a) contacting an RNA polymerase with an oligonucleotide template in a reaction mixture comprising an assay buffer and a fluorescent dye capable of binding double-stranded nucleic acid molecules, under conditions in which the RNA polymerase  
25 is active;
- (b) adding a test compound to the reaction mixture;
- (c) measuring the fluorescence of the reaction mixture; and
- (d) determining whether the test compound modulates RNA polymerase activity.
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31. The method of claim 30, wherein the RNA polymerase is a recombinant RNA polymerase.
32. The method of claim 30, wherein the RNA polymerase is the Hepatitis C virus (HCV) polymerase, NS5B.
33. The method of claim 31, wherein the NS5B polymerase is a recombinant NS5B polymerase.
34. The method of claim 33, wherein the recombinant NS5B polymerase comprises an amino acid sequence as set forth in SEQ ID NO: 2.
35. The method of claim 34, wherein the reaction mixture further comprises large unilamellar vesicles.
36. The method of claim 33, wherein the recombinant NS5B polymerase comprises an amino acid sequence as set forth in SEQ ID NO: 4.
37. The method of claim 30, wherein the fluorescent dye is an unsymmetrical cyanine fluorescent dye.
38. The method of claim 37, wherein the unsymmetrical cyanine fluorescent dye is excited at between 475 nm and 495 nm and dye fluorescence is detected at between 518 nm and 542 nm.
39. The method of claim 30, wherein the compound that modulates the RNA polymerase activity is an antagonist of the RNA polymerase activity.
40. The method of claim 30, wherein the compound that modulates the RNA polymerase activity is an agonist of the RNA polymerase activity.